EXHIBIT C

2004

USP 27

THE UNITED STATES PHARMACOPEIA

NF 22

THE NATIONAL FORMULARY

By authority of the United States Pharmacopeial Convention, Inc., meeting at Washington, D.C., April 12–16, 2000. Prepared by the Council of Experts and published by the Board of Trustees

Official from January 1, 2004

The designation on the cover of this publication, "USP NF 2004," is for ease of identification only. The publication contains two separate compendia: The Pharmacopeia of the United States Twenty-seventh Revision, and the National Formulary, Twenty-second Edition.

UNITED STATES PHARMACOPEIAL CONVENTION, INC.
12601 Twinbrook Parkway, Rockville, MD 20852

NOTICE AND WARNING

Concerning U.S. Patent or Trademark Rights

The inclusion in the Pharmacopeia or in the National Formulary of a monograph on any drug in respect to which patent or trademark rights may exist shall not be deemed, and is not intended as, a grant of, or authority to exercise, any right or privilege protected by such patent or trademark. All such rights and privileges are vested in the patent or trademark owner, and no other person may exercise the same without express permission, authority, or license secured from such patent or trademark owner.

Concerning Use of USP or NF Text

Use of the USP–NF is subject to the terms and conditions of the USP–NF License Agreement. Attention is called to the fact that USP and NF text is fully copyrighted. Authors and others wishing to use portions of the text should request permission to do so from the Secretary of the USPC Board of Trustees.

ISSN 0195-7996
ISBN 1-889788-19-8
Printed in Canada by Webcom Limited, Toronto, Ontario

obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively. From the value obtained in the test for *Loss on drying*, calculate the potency on the dried basis. For Insulin derived from a mixture of beef and pork, calculate the total potency as the sum of the potencies of the beef- and pork-derived insulins, determined separately.

Insulin Injection

» Insulin Injection is an isotonic, sterile solution of Insulin. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units.

Packaging and storage—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Labeling—Label it to indicate the one or more animal species to which it is related, as pork, as beef, or as a mixture of pork and beef. If the Insulin Injection is made from Insulin that is purified, label it as such. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Units per mL.

USP Reference standards ⟨11⟩—*USP Insulin RS. USP Insulin (Beef) RS. USP Insulin (Pork) RS. USP Endotoxin RS.*

Identification—The retention time of the insulin peak in the chromatogram of the *Assay preparation* corresponds to the retention time of the appropriate species in the chromatogram of the *Identification preparation*, as obtained in the *Assay*. [NOTE—It may be necessary to inject a mixture of *Assay preparation* and *Identification preparation*.]

Bacterial endotoxins ⟨85⟩—It contains not more than 80 USP Endotoxin Units for each 100 USP Insulin Units.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 7.0 and 7.8, determined potentiometrically.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Zinc content ⟨591⟩: between 10 and 40 µg for each 100 USP Insulin Units of appropriate species.

Limit of high molecular weight proteins—

Arginine solution, Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin*.

Test solution—Quantitatively add 4 µL of 6 N hydrochloric acid per mL of an accurately measured volume of Injection, and mix.

Procedure—Proceed as directed for *Procedure* in the test for *Limit of high molecular weight proteins* under *Insulin*. Not more than 2.0% is found.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase, Identification preparation, Standard preparation, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Insulin*.

NOTE—The *Identification preparation, Standard preparation,* and *Assay preparation* may be stored at room temperature for up to 12 hours or in a refrigerator for up to 48 hours.

Assay preparation 1 (for Injection labeled as containing 40 USP Insulin Units per mL)—Add 2.5 µL of 9.6 N hydrochloric acid per mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix.

Assay preparation 2 (for Injection labeled as containing 100 USP Insulin Units per mL)—Add 2.5 µL of 9.6 N hydrochloric acid per mL of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the test specimen.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Procedure—Separately inject equal volumes (about 20 µL) of the appropriate *Assay preparation*, the *Identification preparation*, and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the *Identification preparation* to identify the insulin peaks. For Insulin Injection prepared from a single species, calculate the potency, in USP Insulin Units per mL, of the Injection taken by the formula:

$$(CD)(\Sigma r_U / \Sigma r_S),$$

in which C is the concentration, in USP Insulin Units per mL, of USP Insulin RS in the *Standard preparation; D* is the dilution factor; and Σr_U and Σr_S are the sums of the areas of the insulin and A-21 desamido insulin peaks obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively. For Injection prepared from a mixture of beef and pork insulins, calculate the total potency as the sum of the potencies of both beef and pork insulins, determined as directed above.

Insulin Human

$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.58
Insulin (human) [*11061-68-0*].

» Insulin Human is a protein corresponding to the active principle elaborated in the human pancreas that affects the metabolism of carbohydrate (particularly glucose), fat, and protein. It is derived by enzymatic modification of insulin from pork pancreas in order to change its amino acid sequence appropriately, or produced by microbial synthesis via a recombinant DNA process. Its potency, calculated on the dried basis, is not less than 27.5 USP Insulin Human Units in each mg. The proinsulin content of Insulin Human derived from pork, determined by a validated method, is not more than 10 ppm. The host cell derived proteins content of Insulin Human derived from a recombinant DNA process, determined by an appropriate and validated method, is not more than 10 ppm. The host cell or vector derived DNA content and limit of Insulin Human derived from a recombinant DNA process that utilizes eukaryotic host cells are determined by a validated method.

NOTE—One USP Insulin Human Unit is equivalent to 0.0347 mg of pure Insulin Human.

Packaging and storage—Preserve in tight containers. Store in a freezer, and protect from light.

Labeling—Label it to indicate that it has been prepared by microbial synthesis or that it is derived by enzymatic modification of insulin from pork pancreas.

USP Reference standards ⟨11⟩—*USP Insulin Human RS. USP Insulin (Pork) RS. USP Proinsulin (Pork) RS. USP Endotoxin RS.*

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Determine the peptide fragments, using the following peptide mapping procedure.

Sulfate buffer—Mix equal volumes of 2.0 M ammonium sulfate and 0.5 M sulfuric acid, and filter.

Enzyme solution—Prepare a solution of *Staphylococcus aureus* V-8 protease in water having an activity of 500 units per mL.

HEPES buffer—Dissolve 2.38 g of HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) in about 90 mL of water in a 100-mL volumetric flask. Adjust with 5 M sodium hydroxide to a pH of 7.5, dilute with water to volume, and mix.

Solution A—Prepare a filtered and degassed mixture of 100 mL of acetonitrile, 700 mL of water, and 200 mL of *Sulfate buffer*.

Solution B—Prepare a filtered and degassed mixture of 400 mL of acetonitrile, 400 mL of water, and 200 mL of *Sulfate buffer*.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system* Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard digest solution—Dissolve about 6 mg of USP Human Insulin RS in 3 mL of 0.01 N hydrochloric acid, and transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 hours. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

Test digest solution—To 1 mg of Insulin Human add 500 µL of 0.01 N hydrochloric acid, and mix to dissolve. Proceed as directed for *Standard digest solution*, beginning with "Add 2.0 mL of *HEPES buffer*".

Chromatographic system (see *Chromatography* ⟨621⟩)—A liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* %	*Solution B* %	Elution
0	90	10	equilibration
0–60	90→30	10→70	linear gradient
60–65	30→0	70→100	linear gradient
65–70	0	100	isocratic
70–71	0→90	100→10	linear gradient
71–86	90	10	re-equilibration

Chromatograph the *Standard digest solution*, and record the peak responses as directed for *Procedure*: the chromatogram of the *Standard digest solution* corresponds to that of the standard chromatogram provided with USP Insulin Human RS. For the chromatogram of the *Standard digest* the tailing factor is not greater than 1.5; and the resolution, *R*, is not less than 3.4 for digest fragments II and III. [NOTE*—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in all insulins; and Fragment III elutes at the same time in insulin derived from beef and pork.]

Procedure—Using the gradient program, run a blank. Inject equal volumes of the *Standard digest solution* and the *Test digest solution* into the chromatograph, and record the chromatograms. The chromatographic profile of the *Test digest solution* corresponds to that of the *Standard digest solution*.

Bioidentity—It meets the requirements of the *Bioidentity test* under *Insulin*.

Microbial limits ⟨61⟩—The total bacterial count does not exceed 300 per g, the test being performed on a portion of about 0.2 g, accurately weighed.

Bacterial endotoxins ⟨85⟩—It contains not more than 10 USP Endotoxin Units in each mg.

Loss on drying ⟨731⟩—Dry about 200 mg, accurately weighed, at 105° for 16 hours: it loses not more than 10.0% of its weight.

Related compounds—Proceed as directed for the *Related compounds* test under *Insulin* except to use the following gradient elution program. The program initially calls for isocratic elution for about 36 minutes with a *Mobile phase* consisting of a mixture of 78% *Solution A* and 22% *Solution B*. Following the gradient elution phase, the system is returned to the initial conditions of 78% *Solution A* and 22% *Solution B*. Adjust the composition of the *Mobile phase* so that the retention time of the main insulin human peak is between 15 and 25 minutes. The content of A-21 desamido insulin and of other insulin related compounds is not more than 2.0% each of the total amount of insulin and total related compounds.

* Fragment I consists of amino acids A5 to A17 and B1 to B13. Fragment II consists of amino acids A18 to A21 and B14 to B21. Fragment III consists of amino acids B22 to B30. Fragment IV consists of amino acids A1 to A4. A refers to the A-chain of Insulin Human, and B refers to the B-chain of Insulin Human.

Limit of high molecular weight proteins—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin*. Not more than 1.0% is found.

Other requirements—It meets the requirements for *Zinc content* under *Insulin*.

Assay—

Mobile phase, Standard preparation, Assay preparation, Resolution solution, Chromatographic system, and *Procedure*—Proceed as directed in the *Assay* under *Insulin* except to use USP Insulin Human RS and otherwise substitute Insulin Human for Insulin throughout.

Insulin Human Injection

» Insulin Human Injection is an isotonic sterile solution of Insulin Human in Water for Injection. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

Packaging and storage—Preserve in a refrigerator. Protect from sunlight. Avoid freezing. Dispense it in the unopened, multiple-dose container in which it was placed by the manufacturer.

Labeling—The labeling states that it has been prepared either with Insulin Human derived by enzyme modification of pork pancreas Insulin or with Insulin Human obtained from microbial synthesis, whichever is applicable. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units per mL.

USP Reference standards ⟨11⟩—*USP Insulin Human RS. USP Insulin (Pork) RS. USP Endotoxin RS.*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial endotoxins ⟨85⟩—It contains not more than 80 USP Endotoxin Units for each 100 USP Insulin Human Units.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements under *Injections* ⟨1⟩ and for *pH*, *Zinc content*, and *Limit of high molecular weight proteins* under *Insulin Injection*.

Assay—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Insulin*.

Standard preparation—Prepare as directed in the *Assay* under *Insulin Human*.

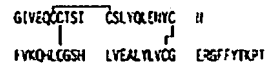
Assay preparations—Prepare as directed in the *Assay* under *Insulin Injection*.

Procedure—Separately inject equal volumes (about 20 µL) of the appropriate *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses for insulin and A-21 desamido insulin. Calculate the potency, in USP Insulin Human Units per mL, of the Injection taken by the formula:

$$(CD)(\Sigma r_U / \Sigma r_S),$$

in which C is the concentration, in USP Insulin Human Units per mL, of USP Insulin Human RS in the *Standard preparation*; D is the dilution factor; and Σr_U and Σr_S are the sums of the areas of the insulin and A-21 desamido insulin peaks obtained from the chromatograms of the *Assay preparation* and the *Standard preparation*, respectively.

Insulin Lispro



$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.58

Insulin (human), 28^B-L-lysine-29^B-L-proline-.

28^B-L-Lysine-29^B-L-prolineinsulin (human) [*133107-64-9*].

» Insulin Lispro is identical in structure to Insulin Human, except that it has lysine and proline at positions 28 and 29, respectively, of chain B, whereas this sequence is reversed in Insulin Human. Insulin Lispro is produced by microbial synthesis via a recombinant DNA process. Its potency is not less than 27.0 USP Insulin Lispro Units per mg, calculated on the dried basis. The proinsulin content of Insulin Lispro, determined by an appropriate and validated method, is not more than 10 ppm. The host cell-derived protein content, determined by an appropriate and validated method, is not more than 10 ppm.

NOTE—One USP Insulin Lispro Unit is equivalent to 0.0347 mg of pure Insulin Lispro.

Packaging and storage—Preserve in tight containers, protected from light, and store in a freezer.

Labeling—Label it to indicate that it has been prepared by microbial synthesis.

USP Reference standards ⟨11⟩—*USP Endotoxin RS. USP Insulin Lispro RS.*

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Determine the peptide fragments, using the following peptide mapping procedure.

Sulfate buffer, HEPES buffer, Mobile phase, Test digest solution, and *Procedure*—Proceed as directed for *Identification* test *B* under *Insulin Human*.

Standard digest solution—Proceed as directed for *Identification* test *B* under *Insulin Human*, except to use USP Insulin Lispro RS instead of USP Insulin Human RS.

Chromatographic system—Proceed as directed for *Identification* test *B* under *Insulin Human*, except to use the following elution program.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–3	95	5	isocratic
3–30	95→41	5→59	linear gradient
30–35	41→20	59→80	linear gradient
35–40	20→95	80→5	return to initial
40–50	95	5	re-equilibration

The flow rate is about 0.8 mL per minute.

Bioidentity—Proceed as directed for *Bioidentity Test* under *Insulin Assays* ⟨121⟩, except to obtain the first blood specimen at 45 minutes, instead of 1 hour, after the time of injection: meets the requirements.

Microbial limits ⟨61⟩—The total aerobic microbial count does not exceed 100 per g, a portion of about 0.3 g, accurately weighed, being used.

Bacterial endotoxins ⟨85⟩: not more than 10 USP Endotoxin Units per mg, the kinetic-chromogenic method under *Photometric Techniques* being used.

Loss on drying ⟨731⟩—Dry about 300 mg, accurately weighed, at 105° for 16 hours: it loses not more than 10.0% of its weight.

Limit of high molecular weight proteins—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin:* not more than 0.25% is found.

Related compounds—

Solvent—Proceed as directed in the *Assay*.

Solution A—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (82 : 18).

Solution B—Prepare a filtered and degassed mixture of *Solvent* and acetonitrile (50 : 50).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve an accurately weighed quantity of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution containing about 3.5 mg per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% A-21 desamido insulin lispro.

Test solution—Dissolve about 3.5 mg of Insulin Lispro in 1.0 mL of 0.01 N hydrochloric acid. Store this solution for not more than 56 hours in a refrigerator.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–60	81	19	isocratic
60–83	81→51	19→49	linear gradient
83–84	51→81	49→19	linear gradient
84–94	81	19	re-equilibration

Adjust the *Mobile phase* composition and duration of the isocratic elution to obtain a retention time of about 41 minutes for insulin lispro, with A-21 desamido insulin lispro eluting near the start of the linear gradient phase. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between insulin lispro and A-21 desamido insulin lispro is not less than 2.5; and the tailing factor for the insulin lispro peak is not more than 2.0.

Procedure—Proceed as directed for *Procedure* in the test for *Related compounds* under *Insulin:* not more than 1.00% of A-21 desamido insulin lispro is found; not more than 0.50% of any other individual insulin lispro related compound is found; and not more than 2.00% of total impurities, excluding A-21 desamido insulin lispro, is found.

Zinc content ⟨591⟩—Determine the zinc content of about 20 mg of Insulin Lispro, accurately weighed: between 0.30% and 0.60% is found, calculated on the dried basis.

Assay—

Solvent—Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, mix, and adjust with phosphoric acid to a pH of 2.3.

Mobile phase—Mix 745 mL of *Solvent* and 255 mL of acetonitrile. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve an accurately weighed quantity of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 1 mg per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% A-21 desamido insulin lispro.

Standard preparation—Dissolve an accurately weighed quantity of USP Insulin Lispro RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 0.7 mg per mL.

Assay preparation—Dissolve an accurately weighed portion of Insulin Lispro in 0.01 N hydrochloric acid to obtain a solution having a concentration of about 0.8 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 10-cm column that contains packing L1. The column temperature is maintained at 40°, and the flow rate is about 0.8 mL per minute. Adjust the *Mobile phase* to provide a retention time of about 24 minutes for the main insulin lispro peak. Chromatograph three replicate injections of the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R*, between insulin lispro and A-21 desamido insulin lispro is not less than 3.0; the tailing factor for the insulin lispro peak is not more than 1.5; and the relative standard deviation for replicate injections is not more than 1.1%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the potency, in USP Insulin Lispro Units per mg, on the as-is basis by the formula:

$$(C_S / C_U)(r_U / r_S),$$

in which C_S is the concentration, in USP Insulin Lispro Units per mL, of USP Insulin Lispro RS in the *Standard preparation;* C_U is the concentration, in mg per mL, of Insulin Lispro in the *Assay preparation;* and r_U and r_S are the insulin lispro peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively. From the value obtained in the test for *Loss on drying*, calculate the potency on the dried basis.

Insulin Lispro Injection

» Insulin Lispro Injection is an isotonic, sterile solution of Insulin Lispro in Water for Injection. It has a potency of not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed as USP Insulin Lispro Units in each mL.

Packaging and storage—Preserve in tight, multiple-dose containers, and store in a refrigerator. Avoid freezing. Protect from sunlight. Dispense it in the unopened, multiple-dose container provided by the manufacturer.

Labeling—The labeling states that it has been prepared with Insulin Lispro obtained from microbial synthesis. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Lispro Units per mL.

USP Reference standards ⟨11⟩—*USP Endotoxin RS. USP Insulin Lispro RS.*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial endotoxins ⟨85⟩: not more than 80 USP Endotoxin Units per 100 USP Insulin Lispro Units, the kinetic-chromogenic method under *Photometric Techniques* being used.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

pH ⟨791⟩: between 7.0 and 7.8.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Limit of high molecular weight proteins—

Arginine solution, Mobile phase, Resolution solution, Test solution, and *Chromatographic system*—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Insulin Injection.*

Procedure—Proceed as directed for *Procedure* in the test for *Limit of high molecular weight proteins* under *Insulin:* not more than 1.50% is found.

Related compounds—

Test solution—Acidify each mL of Injection with 3 µL of 9.6N hydrochloric acid.

Solvent, System suitability solution, Mobile phase, Chromatographic system, and *Procedure*—Proceed as directed in the test for *Related compounds* under *Insulin Lispro.* Not more than 1.50% A-21 desamido insulin lispro is found; and not more than 4.00% of total impurities, excluding A-21 desamido insulin lispro, is found.

Zinc content ⟨591⟩: between 14 and 35 µg for each 100 USP Insulin Lispro Units.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Solvent, Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Insulin Lispro.*

Assay preparation—Acidify each mL of Injection with 3 µL of 9.6N hydrochloric acid. Quantitatively dilute a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 20 USP Insulin Lispro Units per mL.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the potency, in USP Insulin Lispro Units, in each mL of the Injection taken by the formula:

$$CD(r_U / r_S),$$

in which C is the concentration, in USP Insulin Lispro Units per mL, of USP Insulin Lispro RS in the *Standard preparation;* D is the dilution factor used to prepare the *Assay preparation;* and r_U and r_S are the insulin lispro peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Isophane Insulin Suspension

» Isophane Insulin Suspension is a sterile suspension of zinc-insulin crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* Linné (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, is not less than 95.0 percent and not more than 105.0 percent of the potency stated on the label, expressed in USP Insulin Units per mL.

Packaging and storage—Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.

Labeling—Label it to indicate the one or more animal species to which it is related, as porcine, as bovine, or as a mixture of porcine and bovine. Where it is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. The label states the potency in USP Insulin Units per mL. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided.

USP Reference standards ⟨11⟩—*USP Insulin (Beef) RS. USP Insulin (Pork) RS. USP Endotoxin RS.*

Identification—It meets the requirements of the *Identification* test under *Insulin Injection.*

Bacterial endotoxins ⟨85⟩—It contains not more than 80 USP Endotoxin Units per 100 USP Insulin Units.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined,* the Suspension being filtered immediately after it has been reduced to a clear solution by the addition of a freshly prepared 1 in 100 solution of ascorbic acid in *Fluid A.*

pH ⟨791⟩: between 7.0 and 7.8, determined potentiometrically.

Zinc content ⟨591⟩: between 10 and 40 µg for each 100 USP Insulin Units.

Insulin in the supernatant—

Test solution—Centrifuge 10 mL of the Suspension at 1500 × *g* for 10 minutes. Use the supernatant.

Procedure—Determine the insulin content of the *Test solution* by a suitable method: the insulin concentration is not more than 1.0 USP Insulin Unit per mL.